

1. Executive Summary

Recent epidemiological studies have shown a strong relationship between particulate pollution and health outcomes, including increased mortality. Determining the ‘causative agent’ in particles responsible for damaging health is the subject of increasing research activity, but many questions remain. A class of biologically active species known collectively as reactive oxygen species (ROS) is a candidate for part of the adverse health effects caused by particle inhalation. In particles, hydrogen peroxide, H_2O_2 , is the dominant ROS, and the only one that can be measured directly. Ambient particulate peroxide levels exceed those expected from gas-particle partitioning by factors of 200-1000 (Arellanes et al., 2006). This indicates particles *generate* peroxide in aqueous solution (Hasson and Paulson, 2003, Arellanes et al., 2006).

In numerous *in vitro* studies, hydrogen peroxide at levels well below those measured in ambient samples has been shown to damage lung epithelial cells (Oosting et al., 1990, Holm et al., 1991, Brown et al., 2004, Li et al., 2002). Recently, an *in vivo* study showed that two-hour exposures to particulate hydrogen peroxide produced symptoms associated with respiratory distress, while gas-phase peroxides or ammonium sulfate particles alone elicited minimal responses (Morio, et al., 2001). Morio et al. (2001) used elevated PM levels and gas phase H_2O_2 in their study in an effort to create an exposure environment with elevated H_2O_2 dissolved in ammonium sulfate particles, but they were not able to measure particulate peroxide concentrations. As part of this project we duplicated the particle generation system used for the *in vivo* study in our laboratory and found that because ambient particles generate H_2O_2 while ammonium sulfate particles do not (they simply take up H_2O_2 from the gas phase according to Henry’s law), the peroxide concentrations used in that study were well within the normal range for particulate peroxide levels in polluted areas. The *in vivo* results combined with our understanding of peroxides in the study’s particles and in ambient air indicates that very short term exposures (2 hours) to peroxide levels generated by ambient particles produces measurable changes in lung physiology related to inflammation and respiratory distress.

At this time, the underlying mechanism by which particles generate H_2O_2 is not known. The most likely candidates are redox reactions mediated by transition metals and quinones (Hasson and Paulson, 2003, Arellanes et al., 2006). Transition metals are generally present in sufficient concentrations that they, especially if combined with organics that prolong the lifetimes of their photo-oxidized forms, could account for the observed generation of H_2O_2 . Quinones are generally

not present in particles at concentrations sufficient to account for the observed H_2O_2 unless there is a suitable but unknown electron donor in the particles.

In this study, particles were collected on Teflon® filters using impactors to create size cuts, for periods of 1-30 hours for ambient samples or minutes for source materials. The filters were extracted within 30 minutes of sampling completion, using pH adjusted water with ethylene diamine tetra-acetic acid (EDTA) added, and gentle agitation. H_2O_2 was monitored in the samples using high pressure liquid chromatography (HPLC) with fluorescence detection.

In order to further understand the phenomenon of H_2O_2 generation by particles and its relevance to particle health effects, we carried out a series of studies to elucidate H_2O_2 generation activity as a function of pH, and in solutions designed to partly mimic physiological fluids. We also monitored the time dependence of H_2O_2 generation both when particles are in solution and when they are aged on a filter after collection.

The pH of the extraction solution has a different effect on coarse and fine mode particles. Coarse mode particles ($>2.5\ \mu\text{m}$) generate maximum H_2O_2 at pHs between 1 and 3; H_2O_2 generation monotonically decreases between pH 3 and 6, and flattens out again above pH 6 up to 8, at a level that is about 30% of the maximum (low pH) value. This behavior is consistent with H_2O_2 production by transition metals, particularly iron, for which the rates of several redox reactions have been observed to be enhanced by low pHs.

In contrast to coarse particles, fine mode particles ($<2.5\ \mu\text{m}$) generated maximum H_2O_2 at pH 4.5, and lower H_2O_2 generation at both lower and higher pHs. H_2O_2 generation at pH 7.4 was about half of the maximum observed at pH 4.5. This behavior may be consistent with contributions from more than one mechanism to H_2O_2 generation, such as a combination of transition metal and quinone activity.

H_2O_2 generation was either unaffected or significantly enhanced by extraction solutions made to partly mimic lung lining fluid or other physiological fluids. Gamble's and Ringer's solutions, two 'physiologically relevant' solutions from the literature, have ionic strengths of 0.15-0.19 M, pHs of 7.4 and a mixture of inorganic salts. In the case of Gamble's solution, sodium citrate and glycine are also added. Ringer's solution produced larger enhancements to H_2O_2 production (relative to extraction in pH 7.4 waters, by factors averaging 6.5 (4.7 to 7.3) and 3.2 (1.8 to 4.2) for the fine and coarse modes, respectively. Gamble's solution had a smaller effect: fine mode particle H_2O_2 production was enhanced by an average factor of 3.4 (with a range of 2 to 4.2), while the coarse mode exhibited no significant change (average 1.2, range 0.5 to 1.5). There was a reasonably large

degree of variability in this response between samples, so it is not clear that this effect will necessarily be the same for all samples. These results, however, point in the direction of significant generation of H_2O_2 by ambient particles *in vivo*.

Ambient particles were aged to investigate the decay of H_2O_2 generation activity. For the first day or so, particles retain 80 to 90% of their initial activity. By 120 hrs, particles exhibited H_2O_2 activity that was less than 10% of the initial value. Measurements of H_2O_2 production by secondary organic aerosols generated in the laboratory suggests that H_2O_2 generation is an even more labile property of these aerosols; H_2O_2 generation dropped to 15% of the original signal after 45 hours. These reductions in H_2O_2 activity highlight the importance of using fresh particles to study the health effects of ROS. Particles older than a week or two presumably induce effects that are due to something other than their intrinsic ability to generate H_2O_2 and ROS.

Once particles are extracted, the rise of H_2O_2 in solution is fastest in the first 15 or so minutes. The rate of H_2O_2 production declines continuously, reaching an inflection point after about 100 minutes and continuing at a low rate for several hours to several days. Eventually, some particles start to consume ROS. As the process is likely a mixture of reaction kinetics and particle dissolution, it is difficult to compare the process to pure reaction kinetics, but it indicates that after inhalation, this aspect of particle toxicity may taper off after a day to several days.

More finely size fractionated aerosols were collected with a Micro Orifice Uniform Deposition Impactor (MOUDI) for longer periods of 24-30 hours. H_2O_2 generation collected with the MOUDI were in good agreement with the results collected with the virtual impactors, consistent with the aging result above, indicating that particles maintain most of their ability to generate H_2O_2 for durations of about a day. Fine, coarse, and ultrafine fractions exhibited noticeably different behavior for most samples. Overall averages of H_2O_2 generation normalized to particle mass are similar between the size cuts for the UCLA site. The ultrafine size cut has a very small mass of particles, and as a result frequently does not produce signals that are above the detection limit.

A variety of source materials and additional test aerosols were analyzed for ROS generation, including fresh particles from conventional diesel, biodiesel, and biogenic secondary organic aerosols generated in the laboratory, and a standard reference material, NIST SRM 1649a. NIST SRM 1649a was collected in the 1970s, and as expected, showed no ROS generation activity. Diesel PM was investigated repeatedly, both from on-road engines on a chassis dynamometer and from a diesel generator, and showed variable activity depending on load. However, in all cases except one, the H_2O_2 generation activity was well below average ambient activities normalized to particle mass.

Because conventional diesel particles are not very soluble, however, it is unclear if analysis of very fresh diesel particles provides a full picture of the ability of diesel particles to generate ROS. A small number of biodiesel PM samples indicate that biodiesel has significantly higher ROS activity, but the difference may be due in part to their increased solubility in the aqueous extraction solution relative to conventional diesel. Biogenic secondary organic aerosols generated in the laboratory exhibit variable, but generally high ROS generation activity, relative to diesel and ambient aerosols.

This report details many measurements made on ambient aerosols, several of which were collected using a pair of virtual impactors (VIs). The impactors were chosen because they accommodate a high sample flow rates of 55 LPM, while making a single size cut at 2.5 μm , which in turn allows a reasonably high measurement time resolution of 1-3 hours. Measurements made in Riverside in August 2005 had shown highly variable fine mode H_2O_2 levels, and sporadic very high levels of copper, zinc and lead, leading us to suspect either an unusual local industrial source of Cu/Zn/Pb, or sample contamination. Initial investigations did not turn up a contamination source, so we continued to use the VIs in some cases. More recently we have determined that the VIs are indeed releasing Cu and Zn, into the fine mode channel. Similar results have been reported in high velocity inlets (Murphy et al., 2004). As a result, several sets of fine mode results are not reported here. They may be reported later if they are corroborated by results from repeated measurements made with new sampling equipment.

Field campaigns were conducted in Riverside at two different sites: a parking lot on the UC Riverside campus downwind of the 60 freeway during August 2005, and an orange grove on the UC Riverside campus upwind of the 60 freeway during July-August 2008. Because of the contamination issue, only coarse mode results are included in this report. Both campaigns point to metals as the cause of ROS generation in the coarse mode. Correlations between H_2O_2 generation and iron content in the aerosols were significant at the parking lot site, and strong in the orange grove. The orange grove results also showed a significant correlation between ROS generation and aluminum, which was not measured in the earlier study. Additionally, while coarse mode H_2O_2 generation per unit mass was lower at the Riverside site than at UCLA or at the 110 freeway site, because of the very high mass loadings in Riverside, total H_2O_2 generation by coarse mode aerosols in Riverside are higher than at UCLA or the 110 Freeway site. In all of the measurements at Riverside, H_2O_2 activity had little or no significant correlation with particle mass, consistent with the notion that chemical composition plays a more significant role in H_2O_2 generation than does particle mass.